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Intestinal Parasites in Children with Lymphohematopoietic Malignancy in Iran, Mashhad

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Background: Intestinal parasitic disease can cause serious complications for Immunosuppressed patients.

Objectives: This study determines the prevalence of intestinal parasites in children, with lymphohematopoietic malignancy in Mashhad, Iran.

Patients and Methods: In this cross-sectional study stool samples were collected from 89 children (53 boys, 36 girls) with lymphohematopoietic malignancies under chemotherapy, between the age of 1 and 18 years (mean age 7.5 years). Three fresh stool samples taken for three consecutive days were examined by direct smear, formalin-ether method, trichrome staining and ELISA test for *Giardia lamblia* coproantigens.

Results: In this study 35.9% of our patients had parasitic infections and the following parasites were identified; *G. lamblia* (the most prevalent parasite in children) 16 (18%), *Entamoeba coli* 6 (6.7%) *Blastocystis hominis* 5 (5.6%) *Iodamoeba butschlii* 2 (2.2%). *Chilomastix mesnili* 1 (1.1%), *Hymenolepis nana* 1 (1.1%) and *Enterobius vermicularis* 1 (1.1%).

Conclusions: With regards to the high incidence of gastrointestinal parasitic diseases and also because of asymptomatic cases of giardiasis, we recommend evaluation of pediatric patients with malignant lymphohematopoietic disease by at least two different diagnostic methods and three rounds of stool examination in order to prevent possible life threatening outcomes. Coproparasitoscopic study for oncologic patients should be performed and anti-parasitic treatment provided before starting chemotherapy to prevent disseminated parasitic infections. The coproantigen-ELISA is especially advantageous in situations where only a single stool sample can be examined.

Keywords: Intestinal Diseases, Parasitic; Hematologic Neoplasms, Children

1. Background

Parasitic diseases continue to be a major cause of morbidity and mortality, with more than 3 billion people infected worldwide. Many of these infections occur in the developing world, where improved measures to prevent infection require considerable investment in the public health infrastructure. The segment of the population with significant defects in the immune system continues to grow (1). Intestinal parasites still remain a major public health problem in our country.

2. Objectives

Immunocompromised patients have been susceptible to gastrointestinal parasitic infections. In Iran there have been many documents on adults but because of limita-

tions regarding malignant children profiles we decided to collect further information on these subjects. The purpose of this cross-sectional study was determining the prevalence of intestinal parasitic infections among lymphohematopoietic malignant children in Mashhad, Iran.

3. Patients and Methods

This cross-sectional study was conducted at the hematology-oncology service of Dr Sheikh Children's hospital affiliated to Mashhad University of Medical Sciences, and Parasitology lab of Imam Reza Hospital of Mashhad, from October 2008 to October 2009. The research included all patients between 1 day to 18 years of age with lymphohematopoietic malignancies who were admitted to the hematology oncology department and underwent chemotherapy. Patients who had used antiparasitic or antibiotic

Implication for health policy/practice/research/medical education:

Immunocompromised patients have been susceptible to gastrointestinal parasitic infections. This study determines the prevalence of intestinal parasites in children, with lymphohematopoietic malignancy.

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drugs were excluded from the study. The protocol was approved by the university's ethical committee. The parents of the patients were interviewed by a nurse to complete a questionnaire on the type of underlying lymphohematopoietic malignancies, clinical symptoms (such as diarrhea, anorexia, abdominal colic, flatulence, fever and weight loss) and duration of treatment, area of residence, contact with animals, source of drinking water, as well as information about the health status of the child.

Stool samples were collected from 89 children with lymphohematopoietic malignancies. Three fresh stool samples taken for three consecutive days were examined by direct smear, formalin-ether method, trichrome staining and ELISA test for *Giardia lamblia* coproantigens. All specimens were also processed according to the immunoenzymatic assay instruction guide (DRG Giardia Antigen, Germany) to detect *G. lamblia* specific coproantigen. The results were read using a plate reader with a 450 nm filter (Awareness, Stat fax 3200). The samples, which yielded a difference in optical density greater than or equal to 0.150 were considered positive, and those with an optical density less than 0.150 were considered negative. Statistical analyses were performed with the Chi square and ANOVA tests. The data were analyzed using the SPSS statistical software (11th version). Statistical significance was set at $P < 0.05$.

4. Results

Of the 89 patients included in the study, 53 (59.6%) were male and 36 (40.4%) female. The subject's age ranged from 1 month to 18 years (mean 7.5 years SD = 3.8). 83 patients had acute lymphoblastic leukemia (ALL), 4 had acute myelogenous leukaemia (AML) and 2 had non-Hodgkin's lymphoma (NHL).

In this study 35.9% of our patients had parasitic infections and the prevalence of infection according to the type of parasite is shown in Figure 1. The most common parasite was *G. lamblia* 16 (18%) and the other parasites were identified as follows: *Entamoeba coli* 6 (6.7%), *Blastocystis hominis* 5 (5.6%), *Iodamoeba butschlii* 2 (2.2%), *Chilomastix mesnili* 1 (1.1%), *Hymenolepis nana* 1 (1.1%) and *Enterobius vermicularis* 1 (1.1%). The age group of 7-8 years showed maximum frequency of *Giardia* infection, followed by 5-6 years. Although the frequency of *Giardia* was more in females than in males, there was no significant difference between them ($P = 0.144$). 64.3% of patients with giardiasis had no symptoms and 35.7% had clinical symptoms. The most common clinical symptoms were as follows: recurrent abdominal discomfort (35.7%), diarrhea (16.3%), flatulence (15.8%), weight loss (50%) and anorexia (10%).

In this study some factors such as water supply and contact with animals was documented, our findings showed that 44.4% of children with giardiasis use unsanitary water supply (wells or water containers) and 22.2% had contact with animals. 14 cases (87.5%) of giardiasis were detected with the ELISA method. The sensitivity and spec-

ificity of this method were 98.6% and 81.3%, respectively.

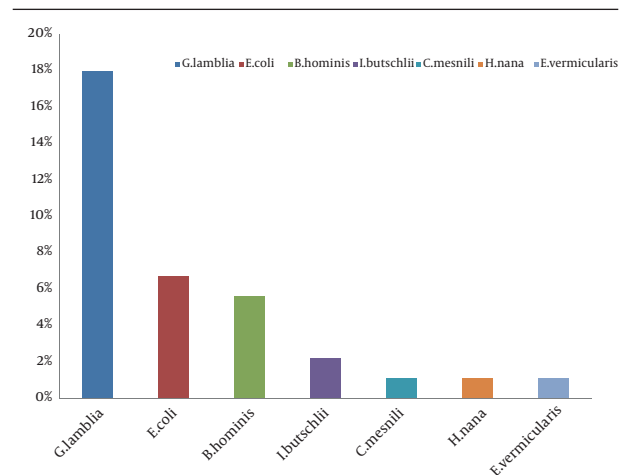


Figure 1. The Prevalence Rate of the Parasitic Infections According to Species of Parasite in Children, with Lymphohematopoietic Malignancy in Mashhad, Iran.

5. Discussion

Parasitic gastrointestinal infections have been variably reported among immune compromised adults in Iran while data on children have been limited. This prospective cross-sectional study aimed to assess the clinical profile of intestinal parasitic infections among lymphohematopoietic malignant children in Mashhad, Iran. Our study demonstrated a prevalence of 35.9% for parasitic infection in lymphohematopoietic malignant children. In Turkey, Aksoy et al. found parasitic infections in 42% of children with neoplasm (1) but in Mexico City Rivera-Luna et al. found parasitosis in 12.9% of childhood acute leukemia (2).

In India, Rudrapatna et al. in a retrospective study found intestinal parasitic infections in 16.5% of patients with malignancy (3). Menon et al. found intestinal parasites in 42% of a group of Malaysian children with cancer (4). Our study shows that the high prevalence rate of parasitic infections may be due to three factors: first, the type of malignancy (lymphohematopoietic malignancies versus other malignant neoplasms); second, immunocompromising effect of the chemotherapy for cancer (all the patients in our study underwent chemotherapy), and third (the most important factor), use of two different diagnostic methods (ELISA in addition to microscopic examination) which led to a higher sensitivity for detection of intestinal parasites.

According to our findings, 18% of patients had giardiasis so that *Giardia* is the most frequently identified enteric parasite in our study; Aksoy et al. found giardiasis in 14% of children with neoplasm (1). However, Rudrapatna et al. reported giardiasis for 3.1% of malignant patients (3). Infection with *G. lamblia* is caused by ingestion of food or water contaminated with cysts. It has worldwide distri-

bution and occurs in developed and developing country (5). *G. lamblia* transmission can be from person to person but is more commonly waterborne, a result of the relative resistance of *G. lamblia* cysts to chlorination. Clinical features of giardiasis vary from the asymptomatic carrier to a severe malabsorption syndrome. Many factors could affect this variation of clinical effects like virulence of the *Giardia* strain, age, host immune system condition and number of cysts swallowed (6).

64.3% of our patients with giardiasis had no symptoms and 35.7% had clinical symptoms. The most common clinical symptoms were recurrent abdominal discomfort (35.7%), diarrhea (16.3%), flatulence (15.8%), weight loss (50%), and anorexia (10%). Although, in our study giardiasis was more frequent in females (56.25%) than males (43.75%) it was not statistically significant ($P = 0.144$).

The age group of 7–8 years showed maximum frequency of *Giardia* infection, followed by 5–6 years. This infection is particularly high in poor and developing countries due to the use of contaminated drinking water, inadequate sanitary conditions and poor personal hygiene (7). In this study, some factors such as water supply and contact with animals have been documented; our findings show that 44.4% of children with giardiasis use unsanitary water supply (wells or water containers) and also 22.2% of children with giardiasis had contact with animals that shows unsanitary conditions. This may indicate that immune compromised children and their parents need health education about prevention of parasitic infections and must also have access to sanitary conditions and good water supply.

Routine stool examinations are normally recommended for the recovery and identifications of intestinal protozoa. However, in the case of *G. lamblia*, because the organisms are attached so securely to the mucosa by the means of a sucking disk, a series of five or six equal stool samples may be examined without recovering the organisms. The organisms also tend to be passed in the stool on a cyclical basis (8). The most important thing about these parasites is that the appearance of *Giardia* in the stool is not so regular and frequent tests are required in order to detect cyst shedding and one direct smear that uses small amounts of sample cannot detect *Giardia* parasites; therefore we need three rounds of stool examination that can increase the chances of diagnosis.

In our study three fresh stool samples taken for three consecutive days were examined by three methods for diagnosis of parasitic infections; direct smear, formalin ether method, trichrome staining and ELISA test for *G. lamblia* coproantigens. Many serological assays have been introduced to detect serum antibodies but because of the lack of appropriate antigens of *Giardia*, the sensitivity of serological tests remains poor. Besides, detection of coproantigen has been successful and commercial kits are available which have been reported to be more sensitive than microscopic diagnosis of giardiasis (9). Pro-

cedures involving the enzyme-linked immunosorbent assay (ELISA) have also been developed to detect *Giardia* antigen in feces. The ELISA is at least as sensitive as microscopic wet examinations (8). Comparison of ELISA test and formalin ether in our study shows that from 16 cases of giardiasis 14 cases can be detected with the ELISA method. The sensitivity and specificity of this method was 98.6% and 81.3%, respectively.

In this study, formalin – ether test considered as a gold standard test and other parasitologic diagnostic methods like direct method and trichrome staining were used and ultimately ELISA for *G. lamblia* antigen detection was tested. It is known that fecal examination to detect *G. lamblia* cysts or trophozoites produces a high percentage of false-negative results. Rocha et al. showed that the assay was able to identify all 30 positive patients (sensitivity = 100.0%). The assay seems to be a good alternative for giardiasis diagnosis, especially when the fecal examination was repeatedly negative and the patient presents symptoms similar to that of giardiasis (10).

The coproantigen-ELISA is especially advantageous in situations where only a single stool sample can be examined. It should not, however, replace microscopic examination of stool specimens for ova and parasites since other potential pathogens would otherwise escape (11). Routine microscopic detection of parasite is inexpensive, but needs expert technicians. Methods for antigen detection can be carried out more quickly and without proficient technicians (12). We highlight the importance of diagnosis and the skills of the laboratory of parasitology, since most parasitic infections responsible for diarrhea in lymphohematopoietic malignant children can be treated. We conclude that because of irregular shedding of *Giardia* and time consuming methods for detection of *Giardia* cysts and trophozoites, it is recommended to use sensitive methods like fecal antigen detection instead of routine methods.

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Authors' Contribution

Study concept and design: Nona Zabolinejad, Fariba Berenji. Zahra Badeii, Abollah Banihashem. Analysis and interpretation of data: Elham Bayati Eshkaftaki, Fariba Berenji, Monavar Afzalaqaei. Drafting of the manuscript: Fariba Berenji. Critical revision of the manuscript for important intellectual content: Fariba Berenji. Statistical analysis: Monavar Afzalaqaei.

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Authors declare that there are not any relevant financial interests related to the material in the manuscript.

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